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Formation and impact of granules in fostering clean energy production and wastewater treatment in upflow anaerobic sludge blanket (UASB) reactors

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A B S T R A C T

Anaerobic reactors have acquired a new relevance in recent years due to their ability to generate methane from biodegradable wastewaters—thereby producing clean energy. Methane capture in this manner also prevents the escape of the greenhouse gas to the atmosphere which otherwise occurs when anaerobic conditions develop in drains and outfalls carrying wastewater. Of all the different types of anaerobic reactors in vogue – anaerobic filter, downflow fixed-film reactor, expanded fluidized-bed anaerobic reactor, etc. – the upflow anaerobic sludge blanket (UASB) reactor is arguably the most widely used. Nearly 80% of the world's anaerobic wastewater treatment systems are estimated to be based on the UASB technology. The functioning of a UASB reactor revolves round its sludge bed which gets expanded as the wastewater is made to flow vertically upwards through it. It is the microflora attached to the sludge particles which acts upon the wastewater. Hence the quality of biofilms sported by the sludge particles, and the intimacy of the sludge–wastewater contact are the factors which, principally, govern the success of a UASB reactor. Very early in the development of UASB technology it was realized that granular sludge of appropriate particle size, particle density, and microfilm characteristics enhances the reactor efficiency in terms of the rate as well as the extent of wastewater treatment. From then onwards efforts have been made by scientists across the world to understand the factors which shape the granules and the manner in which the granules contribute to wastewater treatment. The state-of-the-art is presented in this paper.

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1. Introduction

Between the late 1960s and 1980 several new high-rate anaerobic reactors were introduced: the anaerobic filter [1], the down-flow fixed-film reactor [2], the upflow anaerobic sludge blanket (UASB) reactor [3], and the expanded/fluidized bed anaerobic reactor [4]. The characteristic which distinguishes all these reactors is their ability to carry out anaerobic digestion at much faster rates than was possible earlier. This enables as swift a treatment of wastewaters as was achievable earlier with only aerobic activated sludge process and similar other high-rate aerobic processes. But whereas aerobic processes consume large quantities of energy and produce difficult-to-handle sludges, anaerobic processes enable recovery of a sizable fraction of input energy in the form of methane [5]. Anaerobic processes also yield highly granular and easy-to-handle sludge. By now all these reactors, and their hybrids, have been used in the treatment of various types of biodegradable wastewaters with considerable success but the UASB technology has been by far the most popular. Over 3000 large-scale systems based on this technology are operating across the world [6] with many more in different stages of planning and commissioning.

The compactness of the UASB reactors, their low operational cost, and low sludge production make the UASB technology very attractive. In addition, substantial quantities of 'clean' energy is also generated in UASB reactors in the form of methane. UASB reactors have been particularly successful in the treatment of high-strength industrial wastewaters containing easily hydrolyzable substrates, such as sugar industry wastes, distillery wastes, and brewery wastes [7–10]. The performance of UASB reactors treating difficult-to-hydrolyze and complex substrates such as phenols, effluents from food and milk processing plants, gelatine manufacturing plants, and slaughterhouse wastewaters have been less satisfactory [11–14] but continuous innovations in the UASB design, start-up, and operation have addressed this shortcoming to a large extent [15–21]. Reports have emerged on the successful application of UASB in treating chlorophenols [22,23], azo dyes [24], onion dehydration wastewater [25], high-nitrogen wastewater [26], and ammonium-rich brines [27]. UASB process for the treatment of sewage and other low-strength wastewater has also, traditionally, suffered from a number of shortcomings, such as long start-up time, poor gas production, susceptibility to shock loading, and granule erosion [28,29]. Attempts to solve these problems are now among major initiatives being taken to enhance the reach of the UASB technology [30–34].

It is likely that use of UASB technology may be extended to biohydrogen production as laboratory-scale trials are proving increasingly successful [35–38].

2. Anaerobic digestion process: a brief recapitulation

The web of interactions that occur in the anaerobic digestion process and the associated microflora are reflected in Fig. 1 [39]. The digestion is initiated with the *hydrolysis* step; it involves a wide range of depolymerization and solubilization processes mediated by facultative and obligate fermentative bacteria which facilitate the hydrolysis of the initial proteins and polysaccharides, including the suspended organics present in the wastewater, to monomeric sugars, amino acids, long chain fatty acids and alcohols [40,41]. In the next – acidogenesis – step, further fermentation of the monomeric products by these, and other non-hydrolytic fermentative bacteria, results in the generation of a wide variety of fermentation end products including acetate, formate, methanol, H_2 and CO_2 [42].

The products of acidogenesis are further oxidized to acetate, hydrogen, and carbon dioxide, in a step called *acetogenesis* [43,44]. It is brought about by the obligate hydrogen producing acetogens (OHPAs). Normally, the oxidation of substrates such as butyrate, propionate or ethanol to acetate, hydrogen, and/or formate; or acetate, hydrogen/formate and carbon dioxide; is an endergonic reaction. But when the hydrogen partial pressure gets lowered, for instance due to the presence of hydrogen or formate-utilizing methanogens, the reaction becomes exergonic. Therefore, it is necessary that OHPA bacteria should always grow in syntrophy with hydrogen utilizing methanogens, sulfate-reducing bacteria (SRB), or homoacetogens, in order to facilitate interspecies hydrogen transfer and gain energy from growth on the products

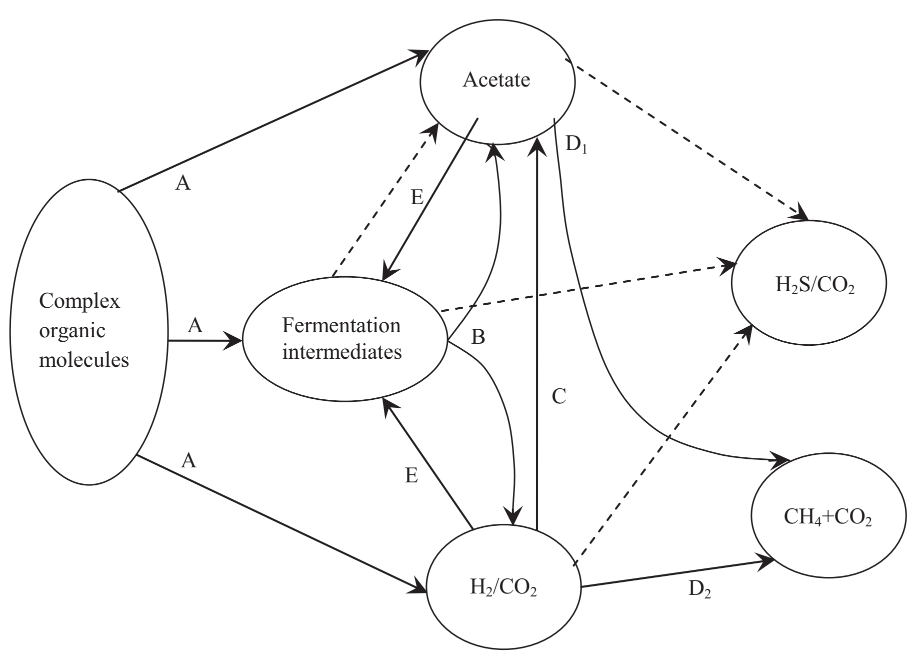

Fig. 1. Web of interactions leading to progressive degradation of complex organic molecules to CH_4, CO_2, and traces of H_2S in a UASB reactor. A: hydrolytic/fermentative bacteria; B: obligate hydrogen producing bacteria; C: homoacetogenic bacteria; D_1: acetoclastic methanogens.
Adapted from [39].

of the acidogenesis phase [43,45]. In this manner OHPAs serve as a link between the initial fermentation stages and the ultimate methanogenic phase.

The final phase is defined by methanogens which are strict anaerobes and have highly diverse cell morphology, ranging from regular and irregular coccoidal cell shape to short rods and long filaments. They may either be hydrogenophilic or hydrogenotrophic species, which form methane by the reduction of H_2/CO_2 or acetoclastic or acetotrophic species, which generate methane by acetate decarboxylation (Fig. 1). Of the two, the acetoclastic methanogens are of greater importance as 70% of the total methane generated during anaerobic digestion is via their mediation [46,47]. It has been shown by Liu et al. [48] that methanobacteriales and methanosarcinales are the main orders of methanogenic populations in anaerobic granular sludge. The finding are based on a phylogenetic analysis of dominant methanogenic populations using mcrA and 16S rRNA target genes in a full-scal UASB treating avernectin wastewater.

In another study by molecular techniques on the microorganisms diversity in a full-scale UASB reactor, treating domestic sewage, it was seen that actinobacteria were dominant at the lower 60% space of the reactor and should have been primarily responsible for the degradation of organic matter [49]. DNA sequences belonging to methanomicrobiales order of Archaea domain were detected in all five levels with the majority producing methane from hydrogen and carbon dioxide.

3. The importance of granulation in UASB reactors

The anaerobic sludge which provides anchorage to microflora is the principle component of a UASB reactor (Fig. 2). It is the continuous interaction of wastewater with the microflora attached with the sludge particles which brings about the treatment achieved in a UASB reactor. Hence the vitality of the microbial films on one hand, and the shape, size, and density of the sludge particles on the other, together, control the efficacy of wastewater treatment in a UASB reactor. The formation of sludge granules becomes exceedingly important because not only do granules support active biofilms but also provide the buoyancy and the settleability necessary to enable very vigorous granule–liquid contact in the reactor.

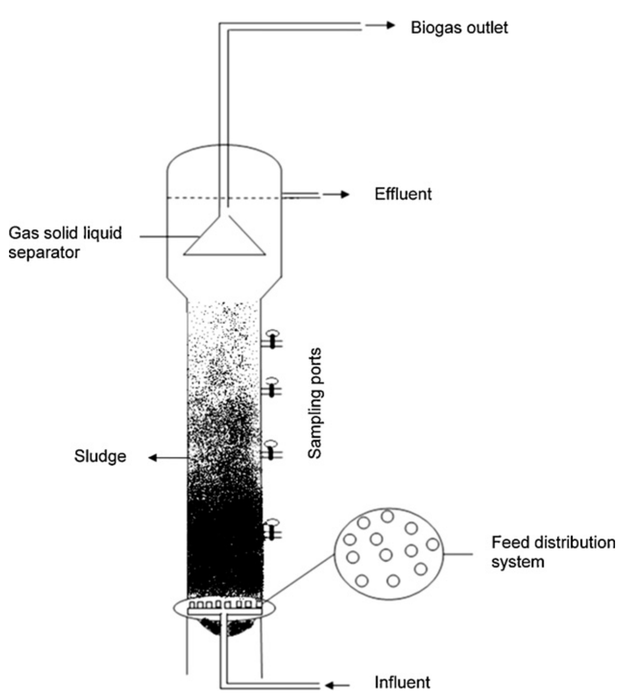

Fig. 2. Schematic of an upflow anaerobic sludge blanket (UASB) reactor.

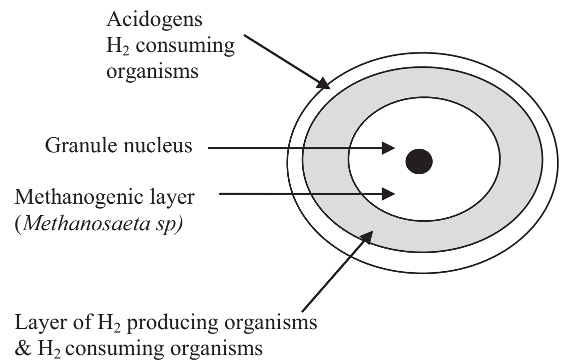

Fig. 3. The proposed layered structure of UASB granules. Adapted from [56].

According to the inventor of the UASB reactor, Lettinga [47], anaerobic granules are particulates enveloped by biofilms, which are formed spontaneously in a UASB reactor by the autoimmobilization of anaerobic bacteria. To put it in other words, granules are dense particles, consisting of an intertwined mixture of symbiotic anaerobic microorganisms. As is well understood now, each granule is a functional unit in itself, comprising of all the different micro-organisms necessary for the methanogenic degradation of organic matter [50,51]. A typical granule is a veritable microecosystem harboring millions of organisms per gram of biomass. However, none of the individual species in these micro-ecosystems are capable of completely degrading influent wastes [52], and associations between the component micro-organisms are necessary [53–56]. A number of techniques have been used, singly and in combinations, to study these syntrophic and competitive interactions within methanogenic granules and to establish links between microbial structure and function. These encompass immunological techniques [57], microscopic, and histochemical techniques [55,58–60]; molecular (rRNA based) methods [50,61,62]; bacterial activity (specific methanogenic activity or SMA) tests [63–65], and/or competition studies.

A layered structure for the granule has also been proposed, in which a central core of acetoclastic methanogens is supposed to be surrounded by a layer of hydrogen- or formate-producing acetogens and hydrogen- or formate-consuming methanogens. The model provides for an outside layer of bacteria that hydrolyse and acidify complex organic matter [50,54,66–68] (Fig. 3).

Once a UASB reactor is seeded with anaerobic sludge and wastewater is made to flow in the upward direction through the sludge, granule formation can slowly occur under appropriate conditions of substrate and nutrient availability, pH, alkalinity, upflow velocity, etc. By-and-by different syntrophic groups come together to form roughly spherically shaped clusters as depicted (in Fig. 4) by McHugh et al. [56]; these clusters have come to be called granules [10,69]. The granules may range from 0.1 to 5 mm in size and possess higher shear strength than flocculated sludge [70,71]. Granulated sludge has better settling property than normal sludge

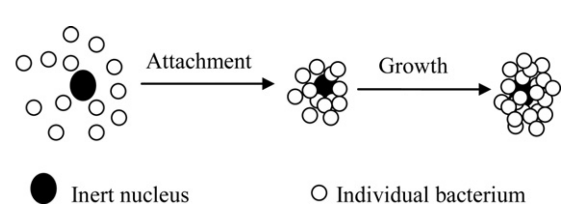

Fig. 4. Granule formation by the attachment of microflora around a nucleus. Adapted from [68].

which allows higher hydraulic loading rates, hence higher reactor efficiency. Granules also reduce inter-species mass transfer limitation between syntrophic groups. Moreover granules can withstand high gas and liquid shear stress without disintegrating; and provide increased resistance to process shocks and toxins compared to dispersed microorganisms [59,70,72–74].

Ideally a granule should contain concentric layers of near-spherical biofilms possessing different bacterial trophic groups [68,75,76]. Each of the trophic groups of bacteria is expected to perform its respective role in the degradation of wastewater, producing biomass and exo-cellular polymers (ECPs) in its vicinity. Among the microflora, the methanogen *Methanosaeta consilii*, is believed to play a key role in setting up granulation, where the clumps formed by growth of these filamentous microorganisms function as nucleation centres that initiate granule development [50,77]. This is followed by subsequent colonization by acetogenic bacteria and hydrogenotrophic methanogens, which often leads to the layered granular biofilm structure reported by several authors [68,76,78–80].

In contrast to the multi-layered structure of methanogenic granules, the hydrogen producing granules (HPGs) of a biohydrogen UASB have simpler matrix comprising mainly of hydrogen-producing acidogens [38]. As acidogens grow quickly, the formation of HPGs is also much quicker than of methanogenic granules.

McHugh et al. [56] have proposed the following list of advantages that the phenomena of granulation offers towards the overall efficiency of the UASB bioreactors:

1. More efficient microbial proliferation.
2. Access to resources and niches that cannot be utilized by isolated cells.
3. Internal physicochemical gradients within the aggregates.
4. Collective defence against antagonists that eliminate isolated cells.
5. Optimization of population survival by differentiation into distinct cell types.
6. Continuous operation of reactors beyond normal washout rates.
7. Generation of a reactor effluent with low suspended solids.
8. Manipulation of biomass in a single phase.
9. Manipulation of growth rates independent of the dilution rate.

The perceived problem of long start-up periods for UASB reactors, due to the time required for anaerobic granulation, may be overcome by the use of flocculants [65,81,82]; or by physical means [191,83].

It is noteworthy that granulation is not always a pre-requisite for successful UASB treatment of some wastes, for example domestic sewage [84]. However, a pretreatment step to remove suspended solids becomes essential if the reactor does not have granular sludge, in order to reach adequate level of treatment efficiency [85–88].

4. Factors which effect granulation: general

Extensive studies have been carried out on the factors which effect granule formation in UASB reactors and influence granule characteristics. In broad terms the factors which play a role include:

- Operating conditions [89–91].
- pH and alkalinity [51,61,92,93].
- Temperature [94,95].
- Strength and composition of wastewater [96–98].
- Reactor hydrodynamics [51,83,95,99].
- Presence of metal ions and trace metals [68,100–103].
- Presence of polymers [70,73,82,104–106].
- Production of exo-cellular polymeric substances by anaerobic bacteria [107–110].

Control of the above-mentioned influences to optimize granule size and density has been the subject of vigorus research during the last 20–25 years. Different attributes are often at cross-purpose. On one hand increase in the granule size causes diffusion to become more rate limiting [72]; on the other hand denser granules cause mass transfer limitations even as they have better settling properties. Sparse granules lose intermediates to convection and have a low settling velocity [10,60]. Hence balancing these and other factors are required to obtain granules of desired characteristics.

Mesophilic granules, as are used in most UASB reactors, are more sensitive to sudden temperature changes than the less oft-used thermophilic granules, resulting in granule disintegration which may, in some cases, lead to reactor failure [111]. Thus, careful temperature control is the first prerequisite for the maintenance of granules in UASB reactors.

5. Role of specific factors in the fashioning of granules

5.1. pH and alkalinity

A stable pH value close to neutrality and a high partial pressure of hydrogen are required to obtain good-quality granulated sludge [92]. The pH values inside a granule have been found to be lower than the bulk liquid [112].

Given that an acidogenic population is significantly less sensitive to pH fluctuations compared to methanogens, which require pH to be in the rather close range of 6.3–7.8, acid formation prevails over methanogenesis when pH is low, resulting in accumulation of volatile fatty acids (VFAs) in the reactor [28,61]. The pH may also change if the un-ionized volatile fatty acid concentration exceeds the buffering capacity of the reactor content [51,113]; this condition is exacerbated due to much more brisk activity of acidogenic bacteria than of the methanogens at lower pH. These conditions can lead to disintegration of the granules because the methanogens may die.

Alkalinity bestows buffering capacity to a UASB reactor [113] thereby providing a hedge against sharp changes in pH. Alkalinity also helps in neutralizing fluctuations in VFA concentration which often arise due to variation in organic loading [114]. Thus, the interplay between alkalinity and chemical oxygen demand (COD) has an influence on granulation [51,92]. Alkalinity levels ranging between 250 and 950 mg/l are considered favorable for the formation and the stability of granules [93].

5.2. Organic loading rate

Organic loading rate (OLR) may very either due to variation in influent COD at a constant flow-rate or variation in flow rate with a constant COD. An OLR at levels beyond its optimum range leads to decrease in pH due to increase in the concentrations of the VFAs [115]. But the methanogens gradually recover and stabilize, consuming the extra VFAs to make the pH rise up again till it stabilizes [98,116]. Low OLR, on the other hand, may starve the microflora and cause other forms of mass transfer limitation leading to disintegration of the larger granules [89,117]. Some authors [104,118,119] have not experienced any granule disintegration even while operating a UASB reactor under very low OLR ($<1.5\ \text{kg COD m}^{-3}\ \text{day}^{-1}$).

There are similarly conflicting reports as to whether granulation occurs or survives during treatment of domestic sewage in UASB reactors. In principal, given the low concentration of readily acidifiable COD present in domestic sewage and the fact that the temperature is normally not optimal for the growth of *Methanosaeta* sp., granulation may seem unlikely, and van Haandel

and Lettinga [28] reported no granulation in any of the UASB reactors treating domestic sewage at full-scale. Other authors [46,120] have reported loss of granular sludge integrity which causes problems of increased sludge washout and decreased reactor efficiency during treatment of domestic sewage and synthetic analogues in UASB reactors. The likely cause of granule disintegration is seen to be low substrate concentration of domestic sewage [46] and mass transfer limitations caused by low upflow velocities and low biogas production. The latter two problems cause the specific methanogenic activity of the reactor sludge to decrease, which in turn leads to internal mass transfer limitation and accumulation of inert organic material at the centre of granules and ultimately granule disintegration [46].

On the other hand several authors have reported that granulation did occur during treatment of both raw and settled domestic sewage [86,87,121]. Furthermore, reports have indicated that granulation proceeds well when EGSB reactors are employed for domestic sewage treatment [85], implying that the formation of granules on domestic sewage may be heavily influenced by the applied upflow velocity. It has also been demonstrated that the process may be enhanced by addition of flocculating agents [73,85,105].

OLR and hydraulic retention time (HRT) can also effect the microbial ecology of granules [122]. An increase in OLR may lead to a shift of *Methanothrix* to *Methanosarcina* in the granules [90]. If the high organic loading is rich in carbohydrates it may cause filamentous bulking of methanogenic granular sludge due to the uncultured filamentous bacteria of the candidate phylum KSB3 [123]. On the basis of rRNA-based activity in a full-scale UASB reactor operated continuously [123], it was revealed that KSB3 cells became active and predominant (up to 54% of the total 16S rRNA) in the sludge when the carbohydrate loading to the system increased. Batch experiments with a short incubation of the sludge with maltose, glucose, fructose, and maltotriose at relatively low concentrations (approximately 0.1 mM) in the presence of yeast extract also showed an increase in KSB3 rRNA levels under anaerobic conditions [123].

Ghangrekar et al. [97] have recommended that OLR in the range of 2.0–4.5 kg COD m^{-3} day^{-1} is suitable for developing good granular sludge, but, logically, the optimum range of OLR and HRT can only be worked out after considering the strength and composition of substrates, temperature, nutrient concentration, macro-metal concentrations, trace metals concentrations, and anions such as sulfate.

5.3. Upflow velocity and effect of gas formation

The upflow velocity (UFV) of influent [99] and the superficial velocity of biogas [28] have an impact on granules. At UFVs above 1 m/h, the granules may disintegrate due to shear stress, and the resulting fragments may wash out of the reactor [99]. Vigorous gas evolution at high OLR may cause the bacterial cells to shear-off from granule surface, eroding the granules [124]. Due to these reasons the UFV is generally kept at about 1 m/h in most of the laboratory and industrial scale reactors, although values up to 6 m/h in laboratory scale reactors have been reported [28].

UFVs of upto 10 m/h can be employed in the expanded granular sludge bed (EGSB) reactor, which is a variation of the UASB reactor [125–127]. This enables higher OLR, of up to 40 kg COD m^{-3} day^{-1}, which in turn, enhances gas production; thereby facilitating even more vigorous mixing inside the reactor [20,126].

5.4. Substrate characteristics

Primarily, concentrations of specific substrates (e.g., acetate for methanogens and sucrose for acetogens) at different locations in the UASB reactor govern the presence of different bacterial species in the granules at those locations. The concentration of a substrate in a location within a granule is in turn governed by the intra-granular diffusion and rate of formation (acetate by acetogen) or consumption (acetate by methanogen). Granules form due to the interacting effects of the rate of intragranular diffusion of substrate and the reaction rates of different steps in the degradation.

In UASB reactors fed with lower fatty acids like acetic acid no acidogenic and acetogenic microorganisms are required and only methanogens are needed to complete the anaerobic degradation process. Hence, in such reactors, the granules primarily consist of methanogens [53]. More complex but easily hydrolyzable noninhibitory substrates of higher concentration, i.e., glucose, proteins, sucrose, and brewery wastes lead to multiple-layered granules [68,76,128]. As, the substrate flux is in a radial direction from the bulk towards the centre, the population density of acetogens and acidogens may be more near the outer surface [66]. On the other hand methanogens are expected to be concentrated near the core of the granules [66,75]. In the intermediate, transition, regions both species are found, but if the reactor feed contains lower fatty acids or simple sugars like sucrose, the methanogens may be present in the outer shell as well [79].

As is the case with low OLR, low substrate concentration in the feed may result in substrate limitation at the core of the larger granules as majority of the substrate is utilized near the surface [89,99]. This may result in flotation of large granules due to hollowing of the core [72]. However, for low substrate concentrations, gas production is also low and the intra-granular pore network may not be filled with gas. As a consequence, these pores may be penetrated by substrates, causing additional transfer of substrates to the core region through these channels. This is perhaps the reason why substrate limitation and flotation of the large granules (up to 3 mm) is not observed for the granules grown with low OLR [104,118,129].

However, low treatment efficiency and very little granulation is generally observed with substrates rich in fats, oil, and grease [13,130]. These constituents tend to accumulate on the surface of sludge granules, resulting in the development of a superficial layer of scum and sludge with the consequent biomass washout [131]. The adsorbed lipid layer may produce a hydrophobic environment which may also result in a reduction in the diffusion of water-soluble substrate into the granules.

Nevertheless some researchers have successfully used UASB reactor for treatment of wastewaters rich in fats, oil, and grease [11,20,132,133]. The granules grown on such substrate are reported to be made up of a large number of close-packed but discrete colonies of multicellular filaments, typically *Methanothrix*, in a dense matrix. Granule formation, and good treatment efficiency has also been reported in feeds containing carbon tetrachloride [64], phenol [16], catechol [134], salinity [135] selenium [136], and sulfate [137].

5.5. Nitrogen and phosphorous

Reports on the impact of luxury dozing of nitrogen and phosphorous in the UASB influent on the granulation process are equivocal. Some authors report that spiking the feed with an excess of nitrogen and phosphorus is helpful in the formation of granules during the start-up phase of a UASB reactor, and that the spiking can be discontinued after the start of the granulation process without any deleterious effect on the granule development [92]. Singh et al. [93] have reported that cell growth reduces drastically at a nitrogen concentration of less than 300 mg/l. Nitrogen, phosphorus, and potassium have been also been shown to cushion the effect of shock loading and prevent the flotation of granules [138,139]. But, there are also reports of inhibition of the process of granulation at higher concentrations of these nutrients [140].

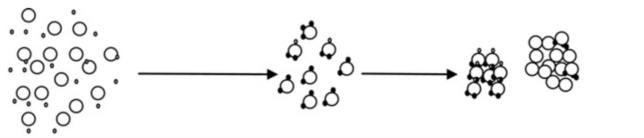

Fig. 5. Role of multivalent cations in granule formation.
Adapted from [68].

Methanogens utilize ammonia as a source of nitrogen, and ammonium-rich substrates can be successfully treated in UASB reactors [27]. Simultaneous presence of high sulfide concentrations in a UASB may support denitrification; at high sulfide levels ammonium may even act as electron donor for N_2 production [141]. These aspects notwithstanding, accumulation of ammonium ions can alter the intracellular pH and the activities of methane-synthesizing enzymes may be inhibited by free ammonia [142].

From the foregoing it is clear that more work is needed before a clear picture can emerge on the concentrations of N and P that are helpful for granule formation and the limits beyond which these elements can exert a deleterious effect.

5.6. Multivalent cations

The UASB granules have a high porosity and a large internal surface area on to which metals can get absorbed at specific sites or just get lodged as insoluble compounds. From the latter form, metals can become labile when pH gets lowered. The presence or absence of several metals, and the concentrations in which they are present, can have profound impact on granule formation [143,144].

The process of granulation is triggered by bacterial adsorption and adhesion to inert particles, to organic precipitates, and/or to each other through physico-chemical interactions and syntrophic associations [68,69,145]. Metal ions evidently facilitate this process by associating with negatively charged groups on cell surfaces (Fig. 5) and causing the linking with exo-cellular polymers [77,65]. In addition, multivalent cations condense the diffused double layers and facilitate flocculation due to van der Waals forces [68,146]. The predominant binding groups for metals on the surface of bacteria are likely to be carboxyl and amino groups in proteins [147]. The solubility of metals is enhanced when the pH drops below 5 hence prolonged exposure of granules to low pH may influence the extent of metal retention within the sludge granules and cause catalytic or inhibitory impacts depending on the concentrations of the metals rendered labile. But thus far the effects of interactions between metals and the granule walls on metal bioavailability and transport have not been clearly understood.

5.6.1. Calcium

Ca^{2+} is one of the nutrients essential to the growth of several strains of microorganisms [93,148]. It has also been found to be effective in speeding up granulation [53,101–103,149–151] and extensively accumulates in microbial aggregates [152]. It has also been reported that beyond certain concentrations the metal may exert a harmful effect, but opinions differ widely on the 'cut-off' limits; it is not clear that exactly at what metal concentrations the beneficial impact peaks off and when the negative impact begins to manifest. For example Jackson-Moss et al. [148] studying the role of Ca^{2+} on a UASB reactor operating on synthetic waste, found that Ca^{2+} concentrations upto 7000 mg/l had no inhibitory effect on digester performance. They also observed that unlike heavy metals which tend to precipitate out and accumulate in anaerobic digesters [153], a large proportion of the Ca^{2+} ions passed through the digester and was present in the effluent. There was, however, some accumulation of Ca^{2+} ions at the base of the digester as a result of precipitation of Ca^{2+} ions as $Ca(OH)_2$. Subsequently Van Langerak et al. [154] reported that under similar biomass yield conditions but with 'less' (6000 mg/l) Ca^{2+} in the influent, 'high' (98%) COD removal efficiencies could be attained while higher (12,000 mg/l) input of Ca^{2+} results in unstable COD removal efficiency as also greater precipitation of the metal as $CaCO_3$ in the reactor.

The acceptable limit of Ca^{2+} was found to be much higher than 1200 mg/l, though much lesser than the one proposed by Jackson-Moss et al. [148], by Johng-Hwa et al. [192]. The latter who, in a study encompassing 0, 1000, 3000, 5000, and 7000 mg/l Ca^{2+} on anaerobic digestion of swine wastewater, found that addition of 3000 mg/l of Ca^{2+} 'gave the best performance'. In contrast to the findings of all these authors, Yu et al. [101] report that Ca^{2+} concentrations upto only 300 mg/l enhanced granulation in a UASB reactor (operated on a 4000 mg/l COD wastewater) and 500 mg/l or higher inputs of Ca^{2+} were detrimental to this cause. In UASB reactors set to generate hydrogen, even 300 mg/l of Ca^{2+} were found to be 'overly high' by Chang et al. [103].

It appears possible that the strengths and characteristics of different UASB feeds play a role in influencing the impact of Ca^{2+} concentration on the granulation and treatment efficiency of UASB reactors. Hence it may be advisable to 'calibrate' different wastewater types for their calcium requirements *vis a vis* granule development for those wastewaters.

As for the manner in which Ca^{2+} assists granulation, it has been suggested by Schmidt and Ahring [72] that the anaerobic granulation process follows the four-step model for biofilm formation:

- transport of cells to the surface of other cells or uncolonised inert material;
- initial reversible adsorption by physico-chemical forces to the substratum;
- irreversible adhesion of the cells to the substratum by microbial appendages and/or polymers; and
- multiplication of the cells and development of the granules.

Evidently, upto certain concentrations calcium accelerates three out of four of these steps:

i) Calcium being a constituent of extracellular polysaccharides may be helpful in the adsorption and linking processes. As calcium precipitates serve as inert supports during granulation, Ca^{2+} might be playing an important role in the initial adsorption.
ii) Calcium may also be promoting adhesion by acting as a link between cells and polysaccharide and between polysaccharide molecules. As bacterial surfaces are usually negatively charged, the presence of divalent cations, like Ca^{2+}, is required to neutralize the charge and serve as a link between components [155].
iii) The presence of calcium can also be associated with the multiplication of cells, in terms of facilitating cell–cell bridging and also indirectly promoting the growth of granules.

5.6.2. Iron

Yu et al. [100] have studied the effect of Fe^{2+} at concentrations 0, 150, 300, 450, 600, and 800 mg/l on the granulation and treatment efficiency of UASB reactors. After 30 days of operation, small granules with diameters of 0.2–0.6 mm became visible at the bottom of reactors spiked with 300–800 mg/l Fe^{2+}. The granules then grew rapidly, and after 30 more days of operation large granules with diameters over 2.0 mm were formed. After about 30 days of rapid growth, the growth rate became slower, indicating that a mature and stable granulation had occurred. The reactor supplemented with 800 mg/l of Fe^{2+} was the first one in which granules became noticeable (on day 24), whereas the control rector was the last to show granules (on day 53). The authors hypothesize that Fe^{2+} might

be accelerating the granulation process through binding between negatively charged groups on cell surfaces and linking extracellular polymers. But the specific methanogenic activity (SMA) of granules decreased with increasing Fe^{2+} concentration in the feed, possibly due to the presence of a large amount of minerals deposited within the granules. The significant decrease in the water content of the granules, and the possible toxicity of high-concentration Fe^{2+} accumulated inside the granules might also have contributed to the decrease in the SMA.

In contrast to the findings of Yu et al. [100], Vlyssides et al. [156,157] have reported all-round beneficial effect of Fe^{2+} when it was fed at the rate of 10 mg/g of COD. As the authors studies influent of COD in the range 2000–10,000 mg/l, this corresponded to Fe^{2+} levels in the influent ranging 20–100 mg/l. It was seen that with the increase in COD loading, and consequently iron loading, in the reactor there was an increase of iron concentration in the reactor sludge. The concentration of iron in granules corresponded directly to their concentration in the feed; the iron was likely retained in the sludge as iron sulfide (FeS) precipitate. The addition of ferrous iron induced a 'stable and excellent' COD conversion rate. As the COD load increased from 2.0 to 10.0 g COD/l day, the settling characteristics of sludge also improved while in the control reactor they did not alter. It became possible to increase liquid upflow velocities in the iron-fed reactor due to its sludge bed having well-defined, larger, granules capable of resisting high hydraulic pressures.

Beneficial effect of iron is also underscored in two recent studies exploring the role of zero-valent iron (ZVI). Lin et al. [158] have investigated the effect of ZVI on the anaerobic biotransformation and dechlorination of chloronitrobenzenes (3,4-DClNB and 4-ClNB). The UASB reactor which had 30 g/l ZVI added to it, successfully developed granular sludge (ZVI-AGS) composed of bacteria associated with precipitated $FeCO_3$ and FeS within 5 months. ZVI addition enhanced 3,4-DClNB transformation and dechlorination efficiencies under high 3,4-DClNB loads, and further promoted dechlorination of 4-chloroaniline (4-ClAn) to aniline. Compared with the AGS formed in the control reactor, iron and its corrosion products were observed and colonized with anaerobes such as methanothrix in ZVI-AGS, and the specific transformation rates of 3,4-DClNB and 4-ClNB using ZVI-AGS were improved by 34% and 64%, respectively. The authors [158] feel that abiotic transformation of ClNBs by ZVI, appropriate concentration of iron corrosion products, lower redox potential, were greater hydrogen production and the main factors providing enhanced transformation and dechlorination of ClNBs in the ZVI-AGS reactors.

In another study [48], a ZVI bed with a pair of electrodes was installed in an UASB reactor and the effects of an electric field and ZVI on granulation were investigated in three UASB reactors operated in parallel: the electric field enhanced ZVI-UASB reactor (R1), a ZVI-UASB reactor (R2) and a common UASB reactor (R3). When a voltage of 1.4 V was supplied to reactor R1, COD removal dramatically increased from 60.3% to 90.7% over the following four days, while the mean granule size rapidly grew from 151 μm to 695 μm over the following 38 days. In comparison COD removal was lower and the increase in granule size was slower in the other two reactors (R1 > R2 > R3). The authors [48] believe that the electric field caused the ZVI to more effectively buffer the acidity and maintain a relatively low oxidation-reduction potential in the reactor. In addition, the electric field resulted in a significant increase in ferrous ion leaching and extracellular polymeric substances (EPS) production. These changes apparently benefited granulation and methanogenesis. Scanning electron microscopy images showed that different microorganisms were dominant in the external and internal layers of the R1 granules. Additionally, fluorescence *in situ* hybridization (FISH) analysis indicated that the relative abundance of methanogens in reactor R1 was significantly greater than in the other two reactors. Zhang et al. [159] using a 9-metal broth which included divalent iron (8120 mg/l) have also reported enhancement of biofilm formation with concomitant increase in the treatment efficiency of a metal-spiked UASB reactor compared to the unspiked one.

5.6.3. Aluminium

Al^{3+} has also been reported to have a positive effect on sludge granulation. Yu et al. [102] studied UASB reactors with and without supplementing the feed with 300 mg/l of Al^{3+}. They found that granules began to appear earlier, and grew faster, in aluminium-spiked reactors. Al^{3+} seemed to improve biomass retention and enabled higher rate of COD removal. However, beyond an OLR of 5.3 g COD/l day the specific methanogenic activity (SMA) in the Al^{3+}-spiked reactors dropped slightly while the SMA in the control reactor continued to increase. Evidently the larger granule size in the former began to hinder mass transfer from the granule surface towards the inner bacterial layers; the mass transfer in this case being driven predominantly by diffusion [138,160]. The difference in the extent of sludge granulation among the two reactors was mainly found only at the initial stages (days 1–60). At the final stage (days 130–146), the control reactor behaved very similarly to the Al^{3+} added reactor; the former had a little less biomass concentration but a slightly higher SMA than the latter, resulting in very similar COD removals and effluent volatile suspended solids (VSS) levels in the two reactors. Hence the addition of $AlCl_3$ played an important role only in the initial stage of granulation and its effect was diminished after the development of mature granules.

In a reassessment of the impact of Al^{3+} supplementation at 300 mg/l on the performance of UASB reactor by Boonsawang et al. [65], it was seen that the reactor with Al^{3+} supplementation could provide a large granule size (0.8 mm) within 35 days, whereas granules of this size in the reactor without Al^{3+} supplement became visible only after 63 days. Moreover, the Al^{3+} spiked reactor could reach steady state within 45 days, 10 days earlier than the control reactor did.

5.6.4. Heavy metals other than iron

Copper, manganese, zinc, cobalt, molybdenum and nickel, besides iron, have been shown to stimulate methane production in anaerobic digesters [161–167,193]. Lack of one or the other of these trace metals has been found to severely limit the overall anaerobic conversion process as well as granulation [143,168–171]. Zhang et al. [144] report that addition of a metallic cocktail containing (in g/l) $MgCl_2{\cdot}6H_2O$ (9.61); $MnSO_4{\cdot}H_2O$ (2.52); KCl (6.58); $CaCl_2{\cdot}2H_2O$ (8.21); $FeCl_2{\cdot}4H_2O$ (8.12); $CoCl_2{\cdot}6H_2O$ (1.4); $NiCl_2{\cdot}6H_2O$ (0.93); $CuCl_2{\cdot}2H_2O$ (0.05); and $ZnCl_2$ (0.05); with 1 g/l of EDTA, caused the treatment efficiency of a UASB reactor treating ethylene glycol increase substantially. Metal-deprived reactors have been reported to stage a recovery when provided with the missing metals [143].

All these findings indicate that several heavy metals are essential for the success of anaerobic digestion but how exactly do metals play this beneficial role is as yet poorly understood [159]. Copper, manganese, zinc, molybdenum, cobalt, nickel, and iron are cofactors for various enzymes [93] and hence may be contributing to the action of microorganisms which contain these enzymes. When Fermoso et al. [143] gave single pulse additions of cobalt(II) in different forms at the rate of 0.25 mg/l to different UASB reactors, they found that cobalt bound to EDTA was hardly retained in the granular sludge but substantial fractions of cobalt in the forms of $CoCl_2$ or cobalt citrate were retained. This happened within the first few minutes after the pulse. The authors speculate that presence of sulfides, carbonates and phosphates in the granules might have caused instantaneous precipitation of cobalt in the granules. There was also a slow retention of cobalt till 24 h after the pulse addition possibly due to chemisorption or ion exchange. Whether happenings like

this influence granule formation is yet to be studied, through it appears likely that they do.

5.7. Vitamins

Bujoczek et al. [172], when operating batch mesaphilic digesters fed with organic fraction of municipal solid waste (OF-MSW), with and without admixture of primary sludge (PS), noted that there was incomplete digestion in reactors fed solely on OF-MSW, and that it was relieved by the addition of PS but not of vitamins. The authors attributed this to the possibility of a shortage of macronutrients like nitrogen in DF-MSW which were compensated by the PS.

Zhang et al. [173] did not study the effect of vitamins on anaerobic sludge development but made an interesting finding that vitamin B_{12} is produced as a byproduct during the anaerobic digestion of alcohol waste fluid in large-enough concentrations (Ca 3 mg/l) to be recoverable economically. Studies of Taconi [174] on the effect of mixtures of trace metals and vitamins on batch-fed anaerobic reactor gave an indication that vitamin supplementation might be helpful in prolonging the usable life of methanogen cells and stimulate higher methane yields.

The first report on the decisive role of vitamins in enhancing anaerobic biodegradation – in this case of chloroform (CF) – has been published by Guerrero-Barajas and Field [175]. They have noted that only negligible degradation of CF was observed in control cultures lacking redox active vitamins, but the addition of riboflavin (RF), cyanocobalamin (CNB_{12}), and hydroxycobalamin enabled biodegradation of CF. The reactions were predominantly catalyzed biologically as evidenced by the lack of any CF conversion in heat-killed controls amended with the cobalamins or minor conversion with RF. In live cultures, significant increases in the rate of CF conversion was observed at substoichiometric molar ratios as low as 0.1–0.01 vitamin: CF for RF and CNB_{12}, respectively.

More recently [176] have reported that a UASB reactor and another reactor designed by the authors both showed a slightly higher methane production when the feed was supplemented with an additive based on vitamins and minerals. Another recent study [143] on the comparative effect of $CoCl_2$ addition and vitamin B_{12} addition (as cobalt source) to a cobalt depleted methanol-fed UASB reactor indicated that the addition of cobalt in the form of $CoCl_2$ supplied enough cobalt to restore methanogenesis and maintain full methanol degradation coupled to methane production during more than 35 days after the $CoCl_2$ pulse. Similar to $CoCl_2$, pulse addition of vitamin B_{12} also supplied enough cobalt to maintain full methanol degradation during more than 35 days after the pulse. However, the specific methanogenic activities (SMAs) of the sludge in the vitamin B_{12} supplied reactor were around 3 times higher than the SMA of the sludge from the $CoCl_2$ supplied reactor at the same sampling times, indicating that vitamin B_{12} may be playing a role more significant than the one of being just a cobalt source.

Reports on the effect of vitamins on photo-biological hydrogen production are equivocal—whereas Mohan et al. [177] found that vitamins were 'not crucial factors' in achieving best results, the same group in a later study [178] state that vitamin solution showed positive influence on both hydrogen production and wastewater treatment irrespective of the experimental variations studied.

The first specific study on the role of vitamins in fashioning the methanogenic activity and settling characteristics of granules in USAB reactors has been carried out by these authors and co-workers [119,179]. It has been shown that the two redox active vitamins – niacin (vitamin B_3) and ascorbic acid (vitamin C) – facilitate speed as well as size of granule formation. This was reflected by favorable size distribution, sludge volume index, and settling velocity of granules in vitamin-augmented UASB reactors compared to controls. The vitamin-spiked reactors also achieved >85% COD removal efficiency in half the number of days the unspiked reactors took. The vitamin supplements were effective at concentrations $\leq$1 mg/l; hence their use in expediting granule formation as also in developing better-quality granules appears economically viable.

5.8. Exo-cellular polymers

Exo-cellular polymers (ECPs) are produced by the bacteria [82] and several studies [59,82,104,108] indicate that the ECPs influence the formation of granules in UASB reactors. It is possible that functional groups associated with the ECP of one microbial cell increase ionic interactions between oppositely charged functional groups in the ECP of other microbial cells, leading to formation of a bond between the two cells. In addition, ions in the media help in bridging between two like-charged functional groups of the cell ECPs [180,181] causing the development of a strong and sticky non-deformable polymeric gel-like matrix [143,182]. However, excessive levels of ECPs do not help the cause of granules and may unhinge floc formation [72]. Interestingly, ECPs isolated from cells cultivated separately and added externally at the startup appear to have no beneficial effect on granulation in the UASB reactors; rather they were seen to exert an inhibitory effect [107].

ECPs bestow a net negative charge on the anaerobic granules and a study by Dignac et al. [183] shows that the proteins, rich in amino acids (such as glutamic and aspartic acid) which also contain carboxylic groups, will contribute to the negative charge of the flocs. A number of researchers have since studied the role of cations in biogranulation and/or bioflocculation processes [146].

5.8.1. Natural and synthetic polymers

Polymers can either immobilize the anaerobic sludge or reinforce the strength of the already existing granules by coating the granule surface with a thin layer of polymer [68,184]. Both synthetic and natural polymers have been used to assist anaerobic bacteria to aggregate together and form granules (Fig. 6); for example the extract of *Moringa oleifera* seeds (a natural polymer) in the feed favors aggregation of coccoid bacteria and growth of microbial nuclei [85]. Cationic polymers which have been seen to enhance granulation include chitosan [104–106,185], the cationic fraction of *Sapindus trifoliata* extract [104], synthetic polymers AA 184 H [70] and Percol 763 [185]. The efficiency of chitosan has been found to be better than the synthetic polymer Percol 763 [185] and the *S. trifoliata* extract [104], but the results are not consistent and some authors [186] have found polymer-assisted granules to be smaller than those of a granular sludge. This has been attributed to the polysaccharide structure of chitosan, which is similar to ECP [187] and the water-absorbing nature of the polymer. Imai et al. [188] have demonstrated the effectiveness of some water-absorbing polymers in the enhancement of granulation. Some anionic polymers such as anionic fraction of *S. trifoliata* extract and sulfonated-lignin also reportedly increase biomass aggregation [9,104]. Jeong et al. [71] have reported the effectiveness of the organic–inorganic hybrid polymers in very quick formation – within a few minutes – of granules with sewage digester sludge.

In a study to compare the relative efficacy of a bioflocculant, an acrylamide–chitosin graft copolymer, and a cationic polymer on the performance of UASB reactors treating low-strength wastewaters, Wang et al. [73] found that the rates of granulation were enhanced by 50%, 87%, and 75%, respectively, by these additives when compared with the control reactor. Though bioflocculant addition did not lead to the development of as much large-size granules it was demonstrated to be the least inhibitory in enhancing microorganism multiplication and improving microbial metabolic activity. The VSS to suspended solids (SS) ratio and the sludge methanogenic activity of the granular samples in the

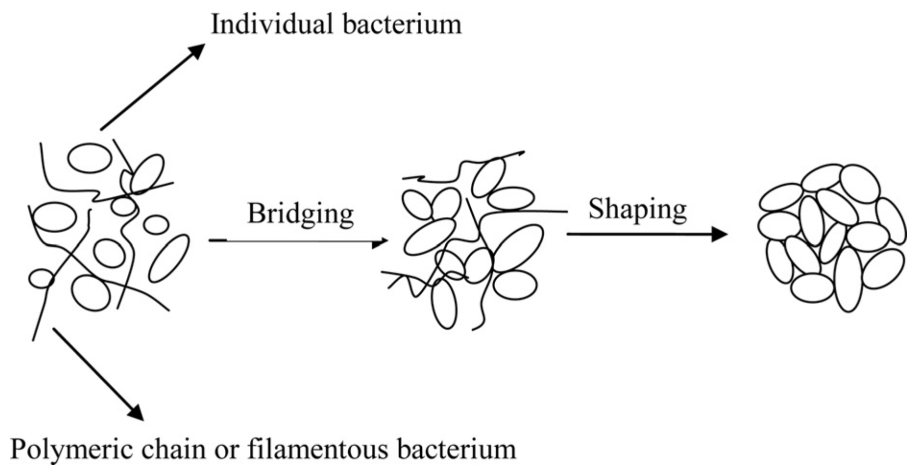

Fig. 6. Polymer-facilitated granule formation.

Adapted from [68].

bioflocculant-spiked reactor were also higher than those of the other three reactors. When compared with the control reactor, flocculant-spiked reactors performed better at most of the organic loading rates as flocculant addition resulted in a considerably higher degree of retention of biomass and lower solids washout from UASB reactors. Granulation was achieved in all four of the reactors, but the granules from flocculant-spiked reactors appeared earlier and were larger than those from the control reactor.

Addition of chitosan, with a degree of deacetylation of 85% and a molecular weight of 2.5×10^5 Da, to a 30-l UASB reactor treating wastewater from a tropical fruit processing industry led to a 24–37% larger granule size and a 6–41% longer solids retention time, compared to control reactor [105]. The reactor performance was also enhanced: the UASB with chitosan addition had a 9–59% lower effluent chemical oxygen demand (COD), 4–10% higher COD removal, up to 35% higher biogas production rate, and a 16–68% lower biomass washout. The difference in performance was statistically significant.

The interactive effect of cationic polymer additives, COD, and UFV was explored by Bhunia and Ghangrekar [51] who studied the influence of these parameters on biomass granulation and COD removal efficiency in a UASB reactor treating low strength wastewater. They formulated statistical models based on these three variables to optimize the biomass granulation and COD removal efficiency in UASB reactors using a two-level factorial design. For the thick inoculums used in the study, having 80 g/l SS, and VSS to SS ratio of 0.3, catinic polymer additives in the inoculums showed adverse effect on biomass granulation and COD removal efficiency. It was concluded that for such thick inoculums, granulation could be achieved even while treating low strength wastewaters by selecting proper combination of influent COD and liquid UFV so as to represent the organic loading rate (OLR) greater than 1.0 kg COD/m^3 day.

Addition of polyaluminium chloride (PAC) too speeds up the development of granular sludge [74]. PAC addition also increases the system resistibility to change in operational conditions and there is no negative impact on microbial activity.

Interestingly clay and sand have also been tried as anchors [189,194] but seemed to have a harmful or no effect on the formation of granular sludge.

Other factors which have been seen to favor granulation are biogas-influenced mixing [190] and the gas–liquid–solid separator design [83]; the latter increases the settleability of suspended particles and accelerates the coagulation of colloidal particles due to the velocity gradient.

6. Summary and conclusion

Of all the high-rate anaerobic reactors, the upflow anaerobic sludge blanket (UASB) reactor has been the most popular by far, with an estimated 80% of all anaerobic wastewater treatment in the world being done with UASB technology. The heart of a UASB reactor is its sludge because the microbial films which bring about wastewater treatment are supported by the sludge particles. By proper manipulation of feed strength, reactor hydraulics, nutrients supplementation and other factors, the sludge particles can be transformed into granules. In turn the granules significantly improve the reactor efficiency by providing more active biofilms and better substrate–microorganism contact. With their resilience and better settling characteristics the granules also confer upon the reactor a greater ability to withstand shock-loads.

An extensive assessment of the past work on the mechanism of granule formation, the factors that influence it (and are influenced by it), and the impact of granules on treatment efficiency reveals the following [145]:

- Composition of granules in a UASB reactor strongly depends on the operational temperature as different species achieve optimum growth rates at different temperatures. Sudden temperature changes could result in granule disintegration in the reactor.
- Optimum alkalinity is essential to maintain the reactor pH and buffer significant fluctuations in the VFA concentrations.
- High partial pressure of hydrogen and neutral pH favor granulation. Changes in OLR and HRT influence these parameters but the optimum range of OLR and HRT can only be decided after considering the influent characteristics and other operating conditions.
- The granule microstructure, especially the layer geometry, is governed by the substrate type and strength.
- The presence of adequate concentrations of bioavailable nutrients and certain metals is essential for granulation.
- Calcium and iron may enhance granulation but are also capable of causing mass transfer limitations when present in large quantities. The effect of calcium is influenced by phosphate.
- The presence of metals at much higher-than-essential concentrations in the influent can cause granule degeneration.
- Although exo-cellular polymers (ECPs) provide strength to the granules, external addition of ECPs has not been seen to enhance granulation.

- Certain natural and synthetic polymers when added to the UASB feed can enhance granulation. They help in the initial stages of granule formation.
- A major challenge before the UASB technology is to shorten the start-up time of the reactor by speeding up granule formation. Various external additives have shown promising results in this direction; however, most of these studies being limited to laboratory scale reactors, have little utility *vis a vis* large-scale application.

Most of the studies reported so far are indicative of the 'helpful' or 'harmful' influences on granule formation and stability. But it is as yet not possible to give precise recipes or even range of values which will work with all substrates, operating conditions, and reactor capacities. As has been seen again and again in the review, the experience of different authors on where the dividing lines fall between 'helpful' and 'inconsequential', or between 'inconsequential' and 'harmful', levels of any influential parameter have differed. Apparently the cut-offs depend on a combination of factors involving type of substrate, OLR, reactor hydrology, HRT, etc., and may have to be ascertained for each specific situation on the basis of the broad directions provided by the state-of-the-art.

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